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(54) Agents and methods for diagnosis and therapy of cancer and cancer risk assessment

(57) The invention relates to agents and methods for the diagnosis and therapy of cancer and further to the identification of cancer associated marker. The following tumor marker were identified: Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endoplasmatic Reticulum ATPase, P20142 Gastricsin (pepsinogen C or II), P07492 Beta-2-Glycoprotein I Precursor (apolipoprotein H), P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11(downregulated in gastric cancer).

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Description

[0001] The invention relates to agents and methods for the diagnosis and therapy of cancer and further to the identification of cancer associated marker. The following tumor marker were identified: Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endoplasmatic Reticulum ATPase, P20142 Gastricsin (pepsinogen C or II), P07492 Beta-2-Glycoprotein I Precursor (apolipoprotein H), P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11(downregulated in gastric cancer).

[0002] The said proteins provide biological marker for diagnosis and therapy of early and late stage carcinomas and methods of cancer risk assessment. The proteins are members of a protein-superfamily which is connected with cell cycle regulation, cell mobility, oxidative stress response and protein folding, protein translocation and protein degradation.

[0003] In spite of improved treatments for certain forms of non-steroid dependent cancer, it is still a leading cause of death in the world. Since the chance for complete remission of cancer is, in most cases, greatly enhanced by early diagnosis, it is very desirable that physicians be able to detect cancers before a substantial tumour develops. Also, in cases where the primary tumour has been substantially removed by surgery or destroyed by other means, it is important that the physician be capable of detecting any trace of cancer in the patient (either in the form of residues of the primary tumour or of secondary tumours caused by metastasis), in order that the physician can prescribe appropriate subsequent treatment, such as chemotherapy.

[0004] The quantities of non-steroid dependent cancer cells that must be detected for early diagnosis or following removal or destruction of the primary non-steroid dependent tumour are so small that the physician cannot rely upon physical examination of the cancer site. Moreover, in many cases the cancer site is of course not susceptible to direct visual observation and it is almost always impractical to detect secondary tumours by visual observation, since it is not possible to predict exactly where they are likely to occur. Accordingly, sensitive tests have to rely upon detection of cancer-associated materials, usually proteins, present in body fluids of patients who have, or are about to develop, cancer cells in their bodies. Several diagnostic materials for detection of cancer-associated proteins are available commercially. Tests for alpha-fetoprotein are used to detect primary liver cancer and teratocarcinoma in humans; and carcinoembryonic antigen is used for digestive system cancers, chorionic gonadotropin is employed to detect trophoblast and germ cell cancers; and prostatic acid phosphatase or prostate specific antigen are used to detect prostate carcinoma. These markers are detectable in advanced rather than in early cancer.

[0005] Unfortunately, many of the commercially available tests are only applicable to a narrow range of non-steroid dependent cancer types, and therefore these tests suffer not only from the disadvantage that other types of cancer may be missed but also from the disadvantage that the narrow applicability of the tests means that it may be necessary to run multiple tests on a single patient for diagnostic purposes, a procedure which not only increases the expense of the diagnostic testing but also increases the risk that one or other of the tests may give a false positive result. Accordingly, there is a need for a single diagnostic test able to detect the presence of very small amounts of cells of a wide variety of different non-steroid dependent cancers like gastrointenstinal cancer and especially colon carcinome in humans. The ideal marker would be one that is specific and universal. Such a marker may exist if malignant transformation is associated with the expression of a unique gene product in all kinds of transformed cells.

[0006] At present, there are no effective presymptomatic clinical signs or biomarkers of susceptibility to non-steroid dependent cancer, making early detection a high priority in medical management of the disease. Efforts to discover prognostic indicators have sought correlations between clinical pathological data and various biochemical parameters. Survival of cancer, whether of the gastrointestinal or colon tissue or another target, is increased through recognition of individuals who are at high risk of a disease, as well as early detection, since current therapeutic strategies for early stage disease have a higher cure rate than for diseases at later stages. For this reason, the identification of molecular markers like proteins of oncogenesis will assist in early diagnosis as well as prognostic monitoring of ongoing disease. Furthermore, if such molecular markers comprise mutations or deletions of genes essential for maintaining normal cellular division, such genes may also be developed as therapeutic agents to treat malignant disease.

[0007] Thus, the technical problem underlying the present invention is to provide agents, methods and marker for tumor diagnosis and therapy.

[0008] The present invention solves this problem by using Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P20142 Gastricsin, P07492 Beta-2-Glycoprotein I Precursor, P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor, 075493 CA11, fragments thereof, nucleic acid sequences encoding said proteins and/or recognition agents against said proteins or said nucleic acid sequences and/or fragments thereof for the manufacture of an agent for diagnosis, prophylactic or therapeutic treatment of non-steroid dependent cancer.

[0009] The said proteins are members of a protein-family which is related to cell cycle regulation, cell mobility, oxi-

dative stress response and protein folding, protein translocation and protein degradation. The proteins of the invention that do not occur in nature are isolated. The term isolated as used herein, in the context of proteins, refers to a polypeptide which is unaccompanied by at least some of the material with which it is associated in its natural state. The isolated protein constitutes at least 0.5%, preferably at least 5%, more preferably at least 25% and still more preferably at least 50% by weight of the total protein in a given sample. Most preferably the isolated protein is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated, and yields a single major band on a non-reducing polyacrylamide gel. Substantially free means that the protein is at least 75%, preferably at least 85%, more preferably at least 95% and most preferably at least 99% free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated.

[0010] In the context of the present invention the term recognition agent refers to molecules which interact with proteins or nucleic acid sequences encoding said proteins or fragments thereof. In a embodiment of the present invention the recognition agent is for instance a polyclonal or monoclonal antibody, a lectin, an oligonucleotid or an antisense construct. The said proteins, or fragments thereof, may be used to produce polyclonal or monoclonal antibodies, which also may serve as sensitive detection reagents for the presence and accumulation of polypeptides in cultured cells or tissues from living patients; the term patient refers to both humans and animals. The full-length proteins or fragments of the proteins may be used to advantage to generate an array of monoclonal antibodies specific for various epitopes of the proteins, thereby potentially providing even greater sensitivity for detection of the proteins in cells or tissues. Although the recognition agent will conveniently be an antibody, other recognition agents are known or may become available, and can be used in the present invention. For example, antigen binding domain fragments of antibodies, such as Fab fragments, can be used. Also, so-called RNA aptomers may be used. Therefore, unless the context specifically indicates otherwise, the term antibody as used herein is intended to include other recognition agents. Where antibodies are used, they may be polyclonal or monoclonal. Optionally, the antibody can produced by a method so that it recognizes a preselected epitope of said proteins. Polyclonal or monoclonal antibodies immunologically specific for the said proteins may be used in a variety of assays designed to localize and/or quantitate the proteins. Such assays include, but are not limited to: (1) flow cytometric analysis; (2) immunochemical localization of the protein in cultured cells or tissues; and (3) immunoblot analysis; e.g., dot blot, Western blot, of extracts from cells and tissues. Additionally, as described above, such antibodies can be used for the purification of said proteins; e.g, affinity column purification, immunoprecipitation.

[0011] Antibody refers in the context of the present invention to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically bind and recognize an analyte (antigen). Antibodies exist, e.g., as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. The term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA methodologies. An antibody specifically binds to or is specifically immunoreactive with a protein when the antibody functions in a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind preferentially to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein.

[0012] The present invention also relates to use recognition agents for diagnosis, prophylactic or therapeutic treatment of non-steroid cancer: the use comprises providing recognition agent with means for the recognition of epitopes of any one member of the protein-family on the surface of a tumour cell, whether as part of the complete protein itself or in some degraded form such as in the presentation on the surface of a cell bound to a MHC protein.

[0013] In a preferred embodiment of the invention the use of the proteins for diagnosis, prophylactic or therapeutic treatment of non-steroid cancer is characterised by a substance which is capable of stimulation of immune system by activating cytotoxic or helper T-cells which recognise epitopes of any one the proteins in the preparation of a medicament to implement a cell-mediated or humoral immune response against a cell in which any one protein of the protein-family is expressed.

[0014] Subject of the invention is further a method for detecting cancer cells in a sample from a patient, the method comprising providing the sample and detecting the level of one or more proteins selected from the group consisting of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P20142 Gastricsin (pepsinogen C or II), P07492 Beta-2-Glycoprotein I Precursor (apolipoprotein H), P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or fragments thereof 075493 CA11(downregulated in gastric cancer) in the sample, and comparing the level of the one or more proteins with a control level that is representative of a level in a normal, cancer-free patient, wherein an modified level of the

proteins in the sample compared to the level of the proteins in the control sample indicates the presence of non-steroid dependent cancer in the patient.

[0015] The Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P47756 F-Actin Capping Protein Beta subunit and/or P11021 78 kDa Glucose regulated protein precursor are overexpressed in tumor tissue. The P20142 Gastricsin (pepsinogen C or II), P07492 Beta-2-Glycoprotein I Precursor (apolipoprotein H), P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11 (down-regulate in gastric cancer) are underexpressed in tumor tissue. The present invention is also based on the discovery that the proteins or members of said protein-family are present - over- or underexpressed or up- or down-regulated - in a wide range of malignant non-steroid dependent tumours.

[0016] In numerous embodiments of the invention, a method for assessing the presence of non-steroid dependent cancer in a patient will include several steps, including providing a biological sample from the patient, detecting the level of one or more proteins or protein-markers of said protein-family in the sample, and comparing the level of the one or more of said proteins with a control level that is representative of a level in a normal, cancer-free patient. Using such methods, an elevation of marker level in a patient compared to the control level indicates the presence of non-steroid dependent cancer or mesoderm tissue tumour in the patient. The level as used herein can refer to protein level, DNA or RNA level, enzyme activity, the presence of particular isoforms, or any other marker of gene number, expression, or activity. To compare levels of markers means to detect marker levels in two samples and to determine whether the levels are equal or if one or the other is greater. A comparison can be done between quantified levels, allowing statistical comparison between the two values, or in the absence of quantification, for example using qualitative methods of detection such as visual assessment by a human. A control sample refers to a sample of biological material representative of healthy, cancer-free humans. The level of a target in a control sample is desirably typical of the general population of normal, cancer-free humans. This sample can be removed from a patient expressly for use in the methods described in this invention, or can be any biological material representative of normal, cancer-free humans, including cancer-free biological material taken from a human with cancer elsewhere in its body. A control sample can also refer to an established level of a target, representative of the cancer-free population, that has been previously established based on measurements from normal, cancer-free humans.

[0017] In a preferred embodiment of present invention the method comprising contacting the sample with a recognition agent for said member of said protein-family selected from the group consisting of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P20142 Gastricsin (pepsinogen C or II), P07492 Beta-2-Glycoprotein I Precursor (apolipoprotein H), P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor 075493 CA11 (downregulated in gastric cancer) and/or fragments thereof and/or the nucleic acid sequences encoding said proteins and/or fragments and detecting binding of the recognition agent to any one member of the protein-family or the nucleic acid sequences encoding the proteins in the prepared sample as an indication of the presence of cancer cells in the sample.

[0018] Accordingly, an aspect of the embodiment provides a method for the identification of tumour cells, which method comprises the use of a recognition agent, for example an antibody, recognising said proteins to contact a sample of tissues, cells, blood or body product, or samples derived therefrom, and screening for a positive response. The positive response may for example be indicated by an agglutination reaction or by a visualisable change such as a colour change or fluorescence, eg immunostaining, or by a quantitative method such as in the use of radio-immunological methods or enzyme-linked antibody methods.

[0019] The method therefore typically includes the steps of (a) obtaining from a patient a tissue sample to be tested for the presence of cancer cells; (b) producing a prepared sample in a sample preparation process; (c) contacting the prepared sample with a recognition agent, such as an antibody or an antisense-construct, that reacts with the any one member or protein of the said protein-family; and (d) detecting binding of the recognition agent to said proteins, if present, in the prepared sample. The human tissue sample can be from for example the bladder, brain, breast, colon, connective tissue, kidney, lung, lymph node, oesophagus, ovary, pancreas, skin, stomach, testis, and uterus or general tissue derived from the mesoderm, especially epithelial tissue.

[0020] A practicable sample preparation process includes tissue fixation and production of a thin section. The thin section can then be subjected to immunohistochemical analysis to detect binding of the recognition agent to the members of the said protein-famiy. Preferably, the immunohistochemical analysis includes a conjugated enzyme labelling technique. A preferred thin section preparation method includes formalin fixation and wax embedding. Alternative sample preparation processes include tissue homogenization, and preferably, microsome isolation. When sample preparation includes tissue homogenization, a preferred method for detecting binding of the antibody to the different members of the protein-family is Western blot analysis. Alternatively, an immunoassay can be used to detect binding of the antibody to said proteins. Examples of immunoassays are antibody capture assays, two-antibody sandwich assays, and antigen capture assays. Preferably, the immnunoassay is a solid support-based immunoassay. When Western

blot analysis or an immunoassay is used, preferably it includes a conjugated enzyme labelling technique.

[0021] In a particularly preferred embodiment of present invention the binding of the recognition agent to any one member of the protein-family in the sample is detected by immunoblotting or immunohistochemical analysis, radioimmunoassay, Western blot analysis mass spectroscopy or enzyme labelling technique. A preferred method for use in the present invention is immunohistochemical analysis. Immunohistochemical analysis advantageously avoids a dilution effect when relatively few cancer cells are in the midst of normal cells. An early step in immunohistochemical analysis is tissue fixation, which preserves proteins in place within cells. This prevents substantial mixing of proteins from different cells. As a result, surrounding normal cells do not diminish the detectability of the member of the protein-family-containing cancer cells. This is in contrast to assay methods that involve tissue homogenization. Upon tissue homogenization, said proteins from cancer cells are mixed with proteins from any surrounding normal cells present in the tissue sample. The concentration of said proteins are thus reduced in the prepared sample, and it can fall below detectable limits. Immunohistochemical analysis has at least three other advantages. First, it requires less tissue than is required by alternative methods such as Western blot analysis or immunoassay. Second, it provides information on the intracellular localization and distribution of immunoreactive material. Third, information on cell morphology can be obtained from the same thin section used to test for the presence of said proteins. Preferably, when immunohistochemical analysis is employed in the practice of this invention, several thin sections from each tissue sample are prepared and analysed. This increases the chances of finding small tumours. Another preferred antibody method for use in the present invention is Western blot analysis, ie sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblotting. Sample preparation for Western blot analysis includes tissue homogenization, and optionally isolation of microsomes. Western blot analysis has the advantage of detecting immunoreactivity on proteins that have been separated with high resolution, according to apparent molecular weight. Inmunoassays such as antibody capture assays, two-antibody sandwich assays, and antigen capture assays can also be used in the present invention. Sample preparation for immunoassays includes tissue homogenization, and optionally isolation of microsomes. Immunoassays have the advantage of enabling large numbers of samples to be tested relatively quickly, and they offer quantitative precision. Principles and practice of immunohistochemistry, Western blot analysis, and immunoassays are well known. One of ordinary skill in the art can select suitable protocols and carry out immunohistochemical analysis. Western blot analysis, or an immunoassay, in the practice of the present invention.

[0022] In a preferred embodiment of the invention said proteins are detected by determining the copy number of nucleic acids encoding for the proteins or fragments thereof in the sample. Detecting a level of said marker proteins refers also to determining the expression level of a gene or genes encoding a target polypeptide or protein. The copy number of a gene can be measured in multiple ways known to those of skill in the art, including, but not limited to, Comparative Genomic Hybridization (CGH) and quantitative DNA amplification (e.g., quantitative PCR) . Gene expression can be monitored in a variety of ways, including by detecting mRNA levels, protein levels, or protein activity, any of which can be measured using standard techniques. Detection can involve quantification of the level of (e.g., gDNA, cDNA, mRNA, protein, or enzyme activity), or, alternatively, can be a qualitative assessment of the level of a target, in particular in comparison with a control level.

[0023] A preferred embodiment method for detecting the presence of nucleic acids encoding for said proteins in the sample, comprising the steps of:

a.) providing a nucleic acid from a biological material of the sample,
b.) contacting the nucleic acid with a nucleic acid segment of continuous sequence that hybridizes under stringent conditions to a contiguous sequence of any one of said proteins under conditions effective to allow hybridization of substantially complementary nucleic acids; and
c.) detecting the hybridized complementary nucleic acids thus formed.

[0024] Persons skilled in the art will appreciate that nucleotide sequences having sufficient homology to be considered substantially the same are often identified by hybridization to one another under appropriate hybridization conditions.

[0025] An another preferred method for detecting the presence of nucleic acids encoding for any one of the proteins of of said protein-family in the sample, comprising the steps of:

a.) obtaining nucleic acids from the sample suspected of containing any one of said proteins,
b.) isolating nucleic acids from the sample,
c.) contacting said nucleic acids with a nucleic acid segment of continuous sequence that hybridizes under stringent conditions to a contiguous sequence of any one of said proteins under conditions effective to allow hybridization of substantially complementary nucleic acids, and
d.) detecting the hybridized complementary nucleic acids thus formed.

[0026] In a preferred method the sample nucleic acids contacted are located within a cell, wherein the sample nucleic acids are DNA or RNA.

[0027] The present invention also relates to a method of obtaining drugs of potential use in cancer therapy, which comprises screening for or selecting a substance which is susceptible to specific metabolism by any one member of the protein-family, and using that substance as a basis for a non-toxic moiety which can be converted by the metabolism of any one member of the protein-family into a toxic one, which kills or inhibits a tumour cell expressing one of any one member of the protein-family or makes it more susceptible to other agents. This other aspect of the invention lies in the presence of said proteins selectively in tumours which provides a mechanism for the selective targeting of anti-cancer drugs based on metabolism in tumours. Drugs can be designed for, or screened for, specific metabolism by said proteins in tumours whereby this metabolism converts a non-toxic moiety into a toxic one.

[0028] The present invention also relates to a method of obtaining drugs of potential use in cancer therapy, which comprises screening for or selecting a substance which is susceptible to specific metabolism by any one member of the protein-family, and using that substance as a basis for a non-toxic moiety which can be converted by the metabolism of any one member of the protein-family into a toxic one, which kills or inhibits a tumour cell expressing of any one member of the protein-family or makes it more susceptible to other agents. This another aspect of the invention provides for the targeting of cytotoxic drugs or other therapeutic agents, or the targeting of imaging agents, by virtue of their recognition of epitopes of said proteins on the surface of a tumour cell, whether as part of the complete said proteins itself or in some degraded form such as in the presentation on the surface of a cell bound to a MHC protein. The present invention also relates to use the proteins, the recognition agents, nucleic acids and/or fragments thereof for diagnosis, prophylactic or therapeutic treatment of cancer.

[0029] The invention also relates also to a method of treatment of non-steroid dependent cancer, characterised by a modification of the level of the proteins, the recognition agents and/or said nucleic acids and/or fragments thereof.

[0030] In a preferred embodiment the method of treatment of cancer is characterised by a reduction of levels of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P47756 F-Actin Capping Protein Beta subunit and/or P11021 78 kDa Glucose regulated protein and/or fragments thereof.

[0031] In a preferred embodiment of present invention the a method of treatment of non-steroid dependent cancer is characterised by the reduction of levels of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P47756 F-Actin Capping Protein Beta subunit and/or P11021 78 kDa Glucose regulated protein precursor in tumour cells in the preparation of a medicament, wherein the substance comprising an inhibitor or means for producing antisense RNA to decrease the synthesis of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P47756 F-Actin Capping Protein Beta subunit and/or P11021 78 kDa Glucose regulated protein.

[0032] In a preferred embodiment of the method a promoter of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P47756 F-Actin Capping Protein Beta subunit and/or P11021 78 kDa Glucose regulated protein is down-regulated.

[0033] In a preferred embodiment the method of treatment of cancer is characterised by a raising of levels of P20142 Gastricsin, P07492 Beta-2-Glycoprotein I Precursor, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11.

[0034] In another preferred embodiment of the invention, the method of treatment is characterised by the raising of levels of P20142 Gastricsin (pepsinogen C or II), P07492 Beta-2-Glycoprotein I Precursor (apolipoprotein H), P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11(downregulated in gastric cancer) in tumour cells in the preparation of a medicament, wherein the substance comprising a stimulator, copy genes or promoter to increase the synthesis of P20142 Gastricsin (pepsinogen C or II), P07492 Beta-2-Glycoprotein I Precursor (apolipoprotein H), P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11 (downregulate in gastric cancer).

[0035] In a preferred embodiment the method of treatment of tumour is characterised by up-regulation of promoters of P20142 Gastricsin (pepsinogen C or II), P07492 Beta-2-Glycoprotein I Precursor (apolipoprotein H), P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11(downregulated in gastric cancer).

[0036] The invention also relates to a kit for detecting of cancer cells in a sample comprising at least one of said proteins, said recognition agents or said nucleic acids or nucleic acid sequences.

[0037] The discovery that the proteins or members of said protein-family are detectable in a wide range of human non-steroid dependent cancers of different histogenetic types gives rise to diagnostic methods for detecting tumours based on these proteins or the genes of the proteins as marker, and to the possibility of tumour therapies involving the proteins or their antibodies or genes.

[0038] From the foregoing discussion, it can be seen that said protein and antibodies thereto and nucleic sequences

which encoded the proteins can be used in many ways for diagnosis and prognosis of human neoplastic diseases of mesoderm tissue, preferably gastrointestinal tumor. However, one person skilled in the art will appreciate that these tools will also be useful in animal and cultured cell experimentation with respect to various carcinomas. They can be used to monitor the effectiveness of potential anti-cancer agents on cellular proliferation in vitro, and/or to assess the development of neoplasms or other malignant diseases in animal model systems.

[0039] The members or proteins of the said protein-family, antibodies thereto or antisense-sequences, provide a much-needed molecular marker for oncogenesis that will assist in early diagnosis and prognostic monitoring of malignant disease, particularly gastrointestinal cancer. The gens which encoded proteins also will be useful in gene replacement therapy or for the development of other therapeutic agents to treat various forms of malignant disease of mesoderm tissue.

[0040] The following examples are provided to describe the invention in further detail. These examples are intended to illustrate and not to limit the invention.

Example

Detection and Characterization of the protein-markers

First dimension - Isoelectrofocusing (IEF)

Sample denaturation

[0041] Material: human tumor tissue: The cell pellets are denaturated with a volume of denaturation solution which has to be adjusted depending of the pellet size (ratio pellet to buffer ~ 1 to 4). After addition of the denaturation solution the samples are vortexed, sonicated three times 10 seconds using the Sonoplus (Bandelin) and centrifuged for 10 minutes at 4°C with maximal speed. They can then be stored at -20°C.

Protein concentration evaluation

[0042] The protein concentration is determined according to the Bradford assay in accordance with the instructions of Bio-Rad and as described in the attachment.

In-gel sample rehydration

[0043] 100µg of protein samples (analytical 2D-PAGE) or 3mg of protein samples (preparative 2D-PAGE), solubilized in 300µl when working with IPG strips from Bio-Rad (or 350µl when working with the strips from Amersham Biosciences) of the above mentioned solution, are pipetted in the focusing tray. After removing the protection film, the IPG strips are then positioned such that the gel of the strip is in contact with the sample (up side down). The gel and the sample are covered with about 2ml low viscosity oil (Mineral oil from Bio-Rad, Cat.No.163-2129) to avoid evaporation. The strips are rehydrated for 8 hours, a low voltage of 50V being applied.

Focusing

[0044] The strips are focused at 20°C under an increasing voltage from 300V to 3500V during 3 hours, followed by 3 additional hours at 3500V [the voltage can also be linearly increased from 300V to 3500V for 8 hours] , the intensity should be 50µA per strip (2mA max. in total). Whereupon the voltage is increased to 10000V until achieving a volthour-product of 80-100kVh.

[0045] After running, the strips can be frozen in glass tubes at -20°C for several weeks, or used immediately for the second dimension.

Second dimension - SDS-PAGE

Equilibration of the strips

[0046] After the first dimension run the strips are equilibrated in order to resolubilise the proteins reduce-S-S- bonds. Each strip is equilibrated twice during 15 minutes (with shaking) with 10ml of equilibration solution. For the first equilibration step, dithiotreitol (DTT) is added to the above mentioned solution at a concentration of 10mg/ml [to ensure that any reformed disulphide bridges are reduced], whereas iodoacetamide (IAA) at a concentration of 48mg/ml is added to the same solution for the following equilibration step [to alkylate the proteins and to react with any reduced

DTT]. After the equilibration, rinse the IPG strips with deionized water for a second and place them on a piece of filter paper at one edge for a few minutes to drain off excess equilibration buffer.

Running SDS gels

[0047] When working with the **Ettan Dalt II** (Amersham) : Use the Buffer Kit from Amersham (Cat.No.17-6002-36) in accordance to the instructions. Place the strip on the top of a precast gel 12.5 (Cat.No.17-6002-36), as well as a wick (on the right end of the strip) on which protein molecular weight standards (Amerham, Cat.No.RPN5800) are loaded. For the preparation of these standards, mix the markers solution with the loading buffer (1:4), and denaturate for 4 min. at 95°C. Pipette the sealing solution on the top of the gel and press carefully the strip with a spatula to achieve complete contact.

[0048] The migration conditions are the following : 2,5 W/gel for 30 minutes and 19 W/gel (170W maximum) for 5 hours until the bromophenol blue tracking dye is going out of the gel.

[0049] When working with the **MultiCell** or the **Criterion Dodeca Cell** (Bio-Rad): seal hot 0,5% agarose solution on top of an SDS gel, and place quickly the strip on top of this gel. Carefully press the strip with a spatula onto the surface of the SDS gel to achieve complete contact. Allow the agarose to solidify for at least 5 minutes. Molecular weight markers can also be used. The migration conditions are the following : 25 V and 40 mA/gel during 1 hour, and then 600 V and 40 mA/gel during 5 or 6 hours, until the bromophenol blue tracking dye has migrated off the lower end of the gel. Electrophoresis buffer 1X has to be prepared.

Silver Staining

[0050] The following steps are realized at room temperature with the Hoefer Processor Plus :

- Fixation : the gels are fixed in 50% methanol / 5% acetic acid in water overnight.
- The gels are washed for 10 minutes with 50% methanol in water, and additionally for 10 minutes with water to remove the remaining acid.
- Sensitizing : the gels are sensitized by a 1 minute incubation in 0,02% sodium thiosulfate.
- The gels are rinsed twice with distilled water for 1 minute.
- Staining : the gels are incubated in chilled 0,1% silver nitrate solution for 20 minutes.
- The gels are rinsed twice with distilled water for 1 minute.
- Development : the gels are developed in developing solution.
- Stop of the reaction : with a solution of 5% acetic acid in water.

[0051] Silver-stained gels are stored in a solution of 1% acetic acid at 4°C until being analyzed.

Isolation of total RNA

[0052]

- to get good RNA out of samples it is necessary to wear gloves all the time and use only RNAse- and DNAse free tips and tubes or to autoclave them

- we use the Qiagen RNeasy Mini Kit Cat no.74 106

1. before use : add β-Mercaptoethanol to buffer RLT (1µl β-ME/1ml buffer)
add 96% ethanol to buffer RPE as indicated on the bottle

2. add 350µl or 600µl buffer RLT (depending on the pellet,larger pellets might even need more RLT buffer) to lyse the cells

3. homogenize the sample with the rotor stator (30s) or with the sonicator (10s

4. add the same volume of 70% ethanol to the sample and mix by pipetting (do not centrifuge and vortex)

5. apply 700µl of this mix to an Rneasy mini spin column and close it if the volume exceeds 700µl load aliquots onto the column

6. centrifuge for 15s at 10.000rpm (sometimes it is necessary to centrifuge higher or longer - it depends on the sample viscosity and the possibility to get it trough the column)

7. discard the flow-trough and reuse the collection tube

8. add 700µl buffer RW1 (in Kit)

9. centrifuge for 15s at 10.000rpm

10. discard the flow-trough and the collection tube and transfer the column in a new collection tube

11. add 500µl buffer RPE (in Kit)

12. centrifuge for 15s at 10.000rpm/ discard the flow-trough

13. add another 500µl buffer RPE

14. centrifuge for 2min at high speed to get rid of the buffer

15. to elute transfer the column to a new 1,5ml collecting tube and pipet 30-50µl Rnase free water (in Kit) directly on the silica- gel membrane

16. centrifuge for 1min at 10.000rpm

17. repeat step 15.and 16. in the same tube

18. close

Agilent Direct-Label cDNA Synthesis Kit Protocol

[0053] We use the Agilent direct label Kit (Cat no. G2555A) Also we need Cyanine 3-dCTP and Cyanine 5-dCTP from Perkin Elmer (NEL 576/ NEL 577)

- Labeling of total RNA requires 20µg per labeling reaction - the amount of RNA is determined with the bioanalyzer (at least 3 dilutions)
- Prepare one tube for cyanine 3 and one for cyanine 5 for each sample (this is because of the dye-swop: there a two arrays on one chip. Each is hybridizied with a Cy 3 and Cy5 labeled sample. On array A01 (the one next to the barcode) we always put NT labelled with Cy3 plus TU labeled with Cy5. On array A02 the TU sample is labelled with Cy3 and is incubated together with the Cy5 labeled NT sample.)
- Pipet the 20µg of RNA to each reaction tube
- Add 2µl DNA Primer (Agilent Kit) and bring the total sample volume to 50µl in nuclease free water
- Incubate tube at 70°C for 10 minutes
- Place reaction tube on ice for 5 minutes
- During these 15 minutes incubation time prepare the master mix (all in Agilent Kit)
- Master Mix for 1 Chip/ 4 labeling reactions :

  57,4 µl nuclease - free water
  82 µl 5x First strand buffer
  41 µl 0,1 M DTT
  4,1 µl dNTP Mix
  2,05 µl 5mM dCTP

8,2 µl MMLV-RT

- store the Master Mix on ice until use
- add to the reaction tubes 2,5µl of either cyanine 3-dCTP or cyanine 5-dCTP
- pipet 47,5 µl of the Master Mix into each sample tube
- incubate cDNA synthesis reaction at 42°C for 60 minutes
- move the reaction to heating block at 70°C for 10 minutes
- place reaction tube on ice for 5 minutes
- add 2,0 µl RNAse IA (Agilent Kit) to each tube and incubate at room temperature for 30 minutes

Purification of labeled cDNA

[0054]

- We use the QIAquick PCR Purification Kit from Qiagen (Cat no. 28104)
- Add 5 volumes of buffer PB (in Kit) to each reaction tube (we have 100µl in the tubes, so we add 500µl buffer)
- Apply the sample to the Qiaquick column which is placed in a 2ml collection tube
- Centrifuge for 60s at 13000 rpm
- Discard the flow- through and reuse the collection tube
- Add 750µl of 35% guanidine hydrochloride (see solutions) and centrifuge for 60s at 13000rpm
- Discard the flow-through and reuse the collection tube
- Wash the column with 750µl Qiagen's buffer PE (add ethanol to the buffer before use; its indicated on the bottle) and centrifuge for 60s at 13000rpm
- Discard the flow-trough and centrifuge again for 60s at high speed to remove any residual ethanol
- Place the column into a clean collection tube
- Add 30µl Qiagen buffer EB to the center of the column , wait for 1 minute, then centrifuge for 60s at 13000rpm
- Repeat this step with additional 30µl of buffer EB and elute into the same tube, so that the final volume should be approximately 60µl
- Combine the appropriate cyanine 3- cDNA and cyanine 5-cDNA in one tube (that means non-tumour 3 and tumour 5 in one tube and non-tumour 5 and tumour 3 in one tube)
- Dry the solution under vaccum in rotary dessicator (Speed Vac) approximately 60 minutes

Preparation of the Hybridization solution

[0055]

- Resuspend, by gently pipetting, the cyanine-3/cyanine-5 labeled cDNA in 7,5µl nuclease- free water
- Store on ice
- Add to the tubes 2,5µl Deposition Control Target (Operon; Cat no. SP300)
  2,5µl Human Cot-1 DNA (Invitrogen; Cat no.15279-011)
  12,5µl Deposition Hybridization buffer (Agilent Kit)
- incubate at 98°C for 2 minutes
- centrifuge 60s at high speed and then leave the solution at room temperature until use

Hybridization of labeled cDNA to slides

[0056] This step is very important and you have to be very careful, because the microarray surface is very delicate. Any touching will destroy the array.

- Place the slide on the microarray slide template (included with the kit) with the barcode facing down
- Pipet 25µl of the prepared Hybridization mixture onto the left side from the active surface without any air bubbles
- Place a clean coverslip onto the same side and slowly lower the coverslip to allow the hybridization mixture to fill

the whole surface of the array (without air bubbles)

- Place the slide into the hybridisation chamber base (the chamber is in the third drawer next to the sink)
- Pipet in the corners of the base approximately 15µl nuclease-free water
- Place the cover on the top of the base and close the chamber (screw tight)
- Incubate overnight (approximately 17 hours) in a 65°C waterbath

Washing slides

[0057]

- Prepare wash solution 1 and 2 (see solution)
- Pass solution through a 0,2µm sterile filtration unit to avoid dust
- Store the solution at room temperature
- Prepare 4 staining dishes :

  one with solution 1, two with solution 2 and one with water
  And a beaker (500ml) with solution 1

- Remove the hybridization chamber from the waterbath and dry it before loosening the screws
- Remove coverslips by gently dipping the slide in the glass with wash solution 1
- Place the slide in the staining dish with solution 1 , put them on a magnetic stirrer and stir for 10 minutes (medium)
- Transfer the slides into the first dish with wash solution 2 and submerse the arrays, to get rid of the wash solution one
- transfer the slides into the second dish with wash solution 2 and stir for 5 minutes (medium)
- dip the slides into the dish with water
- carry the dish with the slides to the centrifuge and quickly transfer the slides into black plastic racks
- immediately put the racks (with the slides) in the centrifuge and start - everything must be prepared before the drying procedure is started
- centrifuge for 2 minutes at 400g (2000rpm)
- transfer the slide in the scanning box (can be found in the cupboard over the Bioanalyzer) and put it into the carousel - do not put it into the home position which is marked with "H"
- put the carousel in the scanner
  (click the bottom "Agilent scanner" and click "browse " in the . scanner window and Save the data under H/public/ scanner... make a new folder for every scan with the name of the sample and the date)
- After it is turned on the scanner needs about 20-30 minutes to completely warm up. Do not scan until the scanner is ready.
- scan (press "scan" if he is ready, who have to say in what directory he should save the data)
- store the slides in a polypropylene slide box in the dark at room temperature

Quality control (RNA)

[0058]

- For the quality control we use the Agilent Bioanalyzer and the Agilent RNA 6000 Nano Assay Lab chip (Nr. 5064-8229); store at 4°C in the fridge of the RNA lab

- Additionally we need RNA 6000 ladder from Ambion (Cat. No 7152); store at -20°C

Preparing the gel dye mix

[0059]

- Place 400µl RNA gel matrix (red top in the Kit) into the top of the spin filter

- Centrifuge them 10min at 5000rpm

- Place 130µl of the filtered RNA gel matrix in a RNAse free tube and add 2µl of RNA dye concentrate (blue top)

- The rest you can store in the fridge and use it later in the same way with the dye

- Vortex the mix and aliquot into 30µl aliquots
- Protect the gel dye mix from light and store the mix at 4°C
- Use it within one week

Preparing the ladder

[0060]

- Aliquot the ladder into 5µl aliquots and heat one of them for 2min at 70-80°C
  (this heated ladder you can use only for two weeks and then you have to heat another tube)
- Store the ladder aliquots at 4°C

Chip Priming station

[0061]

- The chip priming station has two positions :

  the base plate has to be on position C
  The syringe- clip at the topmost position

  (there is a picture of this in the manual from Agilent in the first drawer under the Bioanalyzer)

Loading the Chip

[0062]

- Before use warm all things to room temperature
- Pipette 9µl of gel- dye Mix at the bottom of the well marked with the black G
- Make sure that the plunger is at 1ml
- Press the plunger until it is held by the syringe clip
- Wait for exactly 30s and then pull back the plunger to the 1ml portion
- Open the Chip Priming station
- Pipette 9µl of gel-dye mix in the other wells marked G
- Pipette in all other wells 5µl of the RNA 6000 Marker (green cap in Kit)
- Pipette 1µl of the ladder at the bottom of the well with the ladder sign
- Pipette 1µl of each sample you have into each of the 12 sample wells
- Place the chip in the adapter of the vortex mixer and vortex for exactly 1 minute
- Place the chip in the Bioanalyzer and start the run within 5 minutes

Running the RNA Assay

[0063]

- Select the assay from the Assay menue (Assay - RNA - Eukaryote total RNA Nano)
- Press start
- When the start box appears you can enter the number of samples
- Press start again
- Now you can complete the sample list with the name and the dilution in a table and press ok
- The chip runs
- Data will be saved automatically in a folder only for the bioanalyzer

Cleaning after an Assay

[0064]

- Open the bioanalyzer and remove the chip
- Place the cleaning chip filled with 350µl RNAse free water into the bioanalyzer
- Close the lid for about 10 seconds
- Open it and remove the cleaning chip and wait another 10 seconds for the water to evaporate
- Close the lid

Checking your RNA results

[0065] Major features of a successful ladder run are:

- 6 RNA peaks
- 1 Marker peak (that's the first one)
- all 7 peaks are well resolved
- if a yellow sign over the ladder line appears, than you have to change the highest setpoint in the settings from 2.0 to 0.5 and press apply
- major features for a successful total RNA run are:
  - 2 ribosomal peaks (with a successful sample preparation at time 39-42 s and 45-50 s)
  - 1 marker peak (at the same position like in the ladder)
- in "results" the bioanalyzer shows the amount of RNA

pH range : 3-6 **Search done on 10 patients**

Samples :
P01.0103_SNCX0000001Z = MD5nt
P01.0104_STCX0000002Z = MD5tu
P01.0105_SNCX0000003Z = MD9nt
P010106_STCX0000004Z = MD9tu
P01.0107_SNCX0000006Z = MD11nt
P01.0108_STCX0000007Z = MD11tu
P01.0112_SNCX0000011Y = MD13nt
P01.0113_STCX0000012Y = MD13tu
P010114_SNCX0000013X = MD17nt

P01.0115_STCX0000014Y= MD17tu
P010116_SNCX0000015Y = MD21 nt
P01.0117_STCX0000016Y = MD21tu
P01.0118_SNCX0000017Y = MD22nt
P01.0119_STCX0000018Y = MD22tu
P01.0143_MD51nt
P01.0144_MD51tu
P01.0145_MD56nt
P01.0146_MD56tu
P01.0147_MD60nt
P01.0148_MD60tu

ms : protein identified by mass spectrometry
ni: not identified by mass spectrometry
no comment : protein not analysed by mass spectrometry
Expression + up-regulated protein
Expression - down-regulated protein
T: expressed only in tumor
N: expressed only in normal

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
7	Gastricsin Precursor	P20142		-	
6	Cathepsin E Precursor / Gastricin precursor (Pepsonogen C)*	P14091/ P020142*		-	both proteins are cleary confirmed with PSD, only 1 or two peptides are matching in PMF
6	down regulated in gastric cancer		9665240	-	same protein than CA1, also observed in pH5-8
6	CA11 protein	Q9NS71		-	same protein than down-regulated protein in gastric cancer
6	Spasmolytic Polypeptide Precursor	Q03403		-	
5	Nucleophosmin	P06748		+	
5	Gastricsin Precursor	P20142		-	
5	Epithelial-Cadherin Precursor	P12830		-	
5	Cathepsin E precursor	P14091		-	
5	not identified			-	
5	Hemoglobin Beta Chain	P02023		-	

(continued)

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
5	Myosin Light Chain Alkali, non muscle isoform / Myosin Light Chain Alkali, smooth-muscle*	P16475 / P24572*		+	matching with same peptides
4	Tropomyosin, Fibroblast non-muscle type* / Tropomyosin Beta Chain, Fibroblast and Epithelial	P07226* / P06468		+	more than one Tropomyosin in DB (with equal matching peptides)
4	ATP Synthase Beta Chain, mitochondrial precursor	P06576		-	
4	not identified			-	
4	not identified			-	
4	Nucleobindin 1 precursor	Q02818		-	
4	ATP Synthase Beta Chain	P06576		-	
4	Myosin regulatory light chain 2, smooth muscle isoform / Myosin regulatory light chain 2, nonsarcomeric*	P24844 / P19105*		+	matching with same peptides
4	Protein Disulfide Isomerase Precursor	P07237		-	
3	not identified			+	
3	Vimentin	P08670		+	
3	Vimentin	P08670		+	
3	not identified			-	
3	Complement Component 1, Q Subcomponent Binding Protein	Q07021		-	
3	ATP Synthase Beta Chain, mitochondrial precursor	P06576		-	
3	Protein Disulfide Isomerase Precursor	P07237			
3	Cytokeratin 20	P35900			

(continued)

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
3	(XM_006710) ATP synthase, H+ transporting, mitochondrial F1 complex / ATP syntase beta chain, mitochondrial precursor*	P06576*	18601447	-	
3	(BC007716) unknown / (AF058954) GTP-specific succinyl-CoA synthetase beta subunit*		14043451/37	-	
3	ATP Synthase Beta Chain	P06576		-	
3	Triacylglycerol Lipase, gastric precursor / ATP Synthase Beta Chain*	P07098 / P06576*		-	
3	Pepsin A precursor	P00790		-	
3	not identified			+	
3	Calgranulin B	P06702		+	
3	not identified			-	
3	Cathepsin E Precursor	P14091		-	
3	ATP Synthase D Chain	O75947		-	
2	not identified			+	
2	Transional Endoplasmic Reticulum ATPase	P55072		+	
2	Enhancer of rudimentary homolog	Q14259		+	
2	F-Actin Capping Protein Beta subunit	P47756		+	
2	Vimentin	P08670		+	
2	Vimentin	P08670		+	
2	Alpha-Soluble NSF Attachment Protein	P54920		+	
2	Cytokeratin 7 Cytokeratin 8	P08729 P05787		+	

(continued)

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
2	not identified			-	
2	Alpha-1 B-Glycoprotein	P04217		-	PSD only low intensity
2	Calnexin Precursor	P27824		-	
2	Cytokeratin 8	P05787		-	
2	Peroxiredoxin 2	P32119		-	
2	Actin, Cytoplasmic 1/ Actin, Cytoplasmic 2*	P02570/P02571* *		-	
2	Actin, Cytoplasmic 1/ Actin, Cytoplasmic 2*	P02570 / P02571 *		-	
2	Protein Disulfide Isomerase Precursor	P07237		-	
2	Cytochrom B5	P00167		-	
2	Pepsin A precursor	P00790		-	
2	(X05606 beta subunit / ATP Synthase Beta Chain*	P06576*	28931	-	
2	Triacylglycerol Lipase, gastric precursor	P07098		-	
2	not identified				
2	Cytokeratin 19, Actin, Cytoplasmic 1*, Actin, Cytoplasmic 2**	P08727, P02570*, P02571*|		+	
2	not identified			+	
2	Actin, Cytoplasmic 1/ Actin, Cytoplasmic 2*	P02570 / P02571*		+	
2	not identified			+	
2	(BC016277) unknown / (AF117615) heme-binding protein*		9622095 / 770	+	both proteins have similar sequences
2	Tropomyosin, Fibroblast non-muscle type	P07226		+	

(continued)

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
2	Collagen alpha 1 (VI) Chain precursor	P12109		+	
2	Cathepsin B Precursor	P07858		+	
2	not identified			-	
2	Cytokeratin 8 8	P05787		-	
2	not identified			-	
2	Cathepsin E Precursor	P14091		-	
2	Aldehyde Dehydrogenase	P05091		-	
2	Immunoglobulin J Chain	P01591		-	
1	Tropomyosin, Fibroblast non-muscle type	P07226		+	
1	Cathepsin D Precursor	P07339		+	
1	ATP Synthase Beta Chain, mitochondrial precursor	P06576		-	
1 .	Protein Disulfide Isomerase Precursor	P07237		+	underexpressed for three other patients
1	Heat Shock Cognate 71 kDa Protein	P11142		+	
1	Proteasome activator hPA28 subunit beta		4506237	+	
1	Vimentin	P08670		+	

pH range : 5-8 **Search done on 10 patients**

Samples :
P01.0075_SNCX0000001Z = MD5nt
P01.0076_STCX0000002Z = MD5tu
P01.0077_SNCX0000003Z = MD9nt
P01.0078_STCX0000004Z = MD9tu
P01.0079_SNCX0000006Z = MD11 nt
P01.0080_STCX0000007Z = MD11tu
P01.0083_SNCX0000011Y = MD13nt
P01.0084_STCX0000012Y = MD13tu
P01.0085_SNCX0000013X = MD17nt
P01.0124_STCX0000014Y = MD17tu
P01.0087_SNCX0000015Y = MD21nt
P01.0088_STCX0000016Y = MD21tu
P01.0089_SNCX0000017Y = MD22nt

P01.0090_STCX0000018Y = MD22tu
P01.0149_MD51nt nt
P01.0150_MD51tu tu
P01.0151_MD56nt
P01.0152_MD56tu
P01.0153_MD60nt
P01.0154_MD60tu

ms :	protein identified by mass spectrometry
ni:	not identified by mass spectrometry
no comment :	protein not analysed by mass spectrometry
Expression +	up-regulated protein
Expression -	down-regulated protein
T:	expressed only in tumor
N:	expressed only in normal

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
8	(AB039886) down regulated in gastric cancer		9665240	-	same protein than CA11, other isoform than in pH3-6
7	Aconitate Hydratase, mitochondrial precursor	Q99798		-	
6	Triacylglycerol Lipase, gastric precursor	P07098		-	
6	CA11 protein	Q9NS71		-	same protein than down-regulated protein in gastric cancer, also observed in pH3-6
5	Triacylglycerol Lipase, gastric precursor	P07098		-	
5	Triacylglycerol Lipase, Gastric precursor	P07098		-	
5	Peroxiredoxin 2	P32119		-	
4	Voltage-dependant Anion_selective channel protein 2	P45880		-	
4	Acyl-CoA Dehydrogenase, medium chain specific	P11310		-	
4	Beta-2-Glycoprotein I Precursor	P02749		-	
4	Hemoglobin Beta Chain	P02023		-	
4	Calgranulin B	P06702		+	

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
4	Cathepsin B Precursor	P07858		+	
4	not identified			-	
4	Superoxide dismutase [Cu_Zn]	P00441		-	
3	Proteasome activator hPA28 subunit beta		4506237	+	
3	Voltage-dependant Anion_selective channel protein 2	P45880		-	
3	Dihydrolipo-amide Dehydrogenase, mitochonrial precursor	P09622		-	
3	Succinate Dehydrogenase [ubiquinone] Flavoprotein	P31040		-	
3	Cytokeratin 20	P35900		-	no typical contamination keratin
3	Delta-Aminolevulinic Acid Dehydratase	P13716		-	
3	Electron Transfer Flavoprotein-Ubiquinone Oxidoreductase	Q16134		-	
3	unnamed protein product		13376741	-	
3	NADH-Ubiquinone Oxidoreductase 42kDa Subunit	O95299		-	
2	Enhancer of rudimentary homolog	Q14259		+	

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
3	Calgranulin A	P05109		+	identification by comparison with spot 1720 from gel P01.0152
3	S 100 calcium-binding protein A14/ Calgizzarin	O60417 / P31949		+	identification by comparison with spot 1647 from gel P01.0150
3	Heat Shock 27kDa Protein	P04792		+	
3	Cathepsin B Precursor	P07858		+	
3	Carbonic Anhydrase I	P00915		-	
3	Hemoglobin Beta Chain / Hemoglobin Delta Chain*	P02023 / P02042*		-	similar sequences
3	not identified			-	
3	Hemoglobin Beta Chain / Hemoglobin Delta Chain*	P02023 / P02042*		-	similar sequences
3	Hemoglobin Beta Chain	P02023		-	
3	Hemoglobin Beta Chain / Hemoglobin Delta Chain*	P02023 / P02042*		-	similar sequences
3	Hemoglobin Beta Chain	P02023		-	
3	Actin, Cytoplasmic 1 / Actin, Cytoplasmic 2	P02570 / P02571		+	
3	Triacylglycerol Lipase, gastric precursor	P07098		-	

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
3	Fumarate Hydratase, mitochondrial precursor	P07954		-	
2	Lamin A/C	P02545		+	
2	Vimentin	P08670		+	
2	78 kDa Glucose-regulated protein precursor	P11021		+	
2	PDZ and LIM Domain Protein 1	O00151.		+	
2	not identified			+	
2	Cathepsin D Precursor	P07339		+	
2	(AE006639) similar to homoproto-catechuate catabolism bifunctional isomerase		14336767	-	
2	glyoxylate reductase / hydroxypyruvate reductase		6912396	-	
2	Fumarase Hydratase, mitochondrial precursor	P07954		-	
2	150kD Oxygen-regulated Protein Precursor	Q9Y4L1		-	
2	Elongation Factor TU, mitochondrial precursor	P49411		-	

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
2	ATP Synthase Alpha Chain, mitochondrial precursor	P25705		-	
2	Catalase	P04040		-	
2	UDP-Glucose-6-Dehydrogenase	O60701		-	
2	Acyl-CoA Dehydrogenase, Short Chain specific	P16219		-	
2	Glutamate Dehydrogenase 1, mitochondrial precursor	P00367			
2	IgG Fc binding protein		4321127	-	
2	Protein Disulfide Isomerase A3 Precursor	P30101		-	
2	Glutaryl-CoA Dehydrogenase, mitochondrial precursor	Q92947		-	
2	Protein Disulfide Isomerase Precursor	P07237		+	
2	(BC005019) S-100type calcium binding protein A14		10190712	+	
2	(BC014923) similar to RIKEN cDAN 2600015J22		NCBI 1745	+	

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
2	Endoplasmic reticulum protein ERp29 precursor / Metaxin 2*	P30040 / O75431*		-	
2	Hemoglobin Beta Chain / 3-hydroxyacyl-CoA dehydrogenase type II*	P02023 / Q99714*		-	
2	NADH-Ubiquinone Oxidireductase 24kDa subunit, mitochondrial precursor	P19404		-	
2	(BC008188) unknown		16158005	-	
2	NADH-ubiquinone oxidoreductase 13 kDa-B subunit	Q16718		-	
2	Cytochrom C Oxidase Polypeptide VIB (AED)	P14854		-	
2	Actin, cytoplasmic 1 / Actin, Cytoplasmic 2*	P02570 / P02571*		+	
2	(XM_034183) capping protein (actin filament)		14733001	+	
2	Lithostathine 1 Alpha precursor	P05451		-	
2	L-Lactate dehydrogenase B chain	P07195		-	
2	Poly(RC)-Binding Protein 1	Q15365		-	

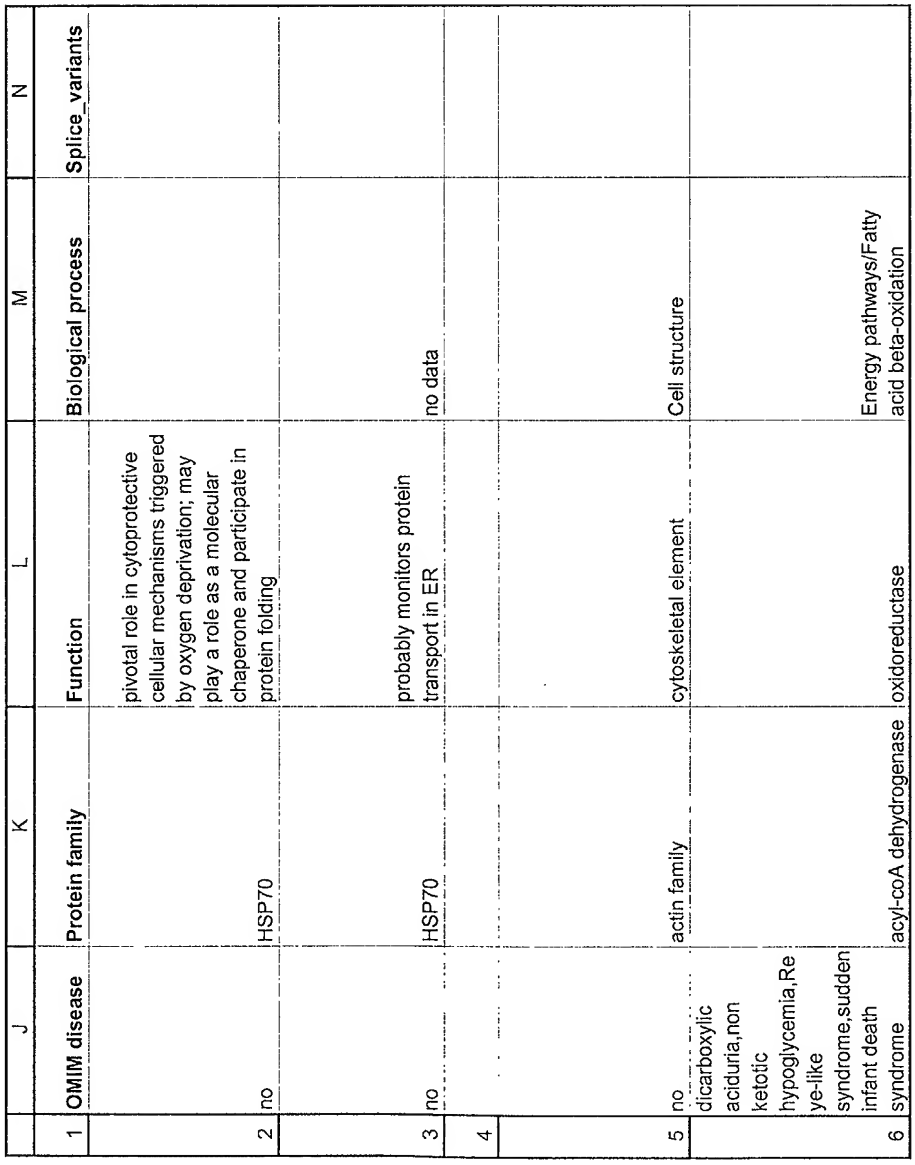
Reproducibility	Name	Swissprot	NCBI	Expression	Comments
2	Tissue Alpha-L-Fucosidase Precursor	P04066		-	
2	Catenin Delta-1	O60716		-	
2	Aldehyde Dehydrogenase 1A1	P00352		-	
2	(M94132) MUC2		186398	-	
2	Acyl-CoA Dehydrogenase, short chain specific	P16219		-	
2	Glycine amidinotransferase, mitochondrial precursor / (BC008202) hypothetical protein FLJ20604*	P50440	8923559*	-	
2	Carbonic Anhydrase II	P00918		-	
2	(BC003409) ovarian carcinoma immunoreactive antigen		8923427	-	
2	78kDa Glucose-regulated protein precursor	P11021		-	
1	(D42041) ha1225 gene product is related to human alpha-glucosidase		577295	+	No entry in SP / multiple entries in NCBI for similar sequences
1	KIAA0088		14773518	+	

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
1	Gelsolin Precursor, Plasma	P06396		+	
1	Actin, Cytoplasmic 1	P02570 P02571		+	
1	Cytokeratin 19	P08727		+	no typical contamination keratin
1	Mitochondrial 28S ribosomal Protein S22	P82650		+	
1	IgG Fc binding protein		4503681	+	high molecular weight protein, result confirmed by 2 PSD spectra (probably fragment of this protein)
1	IgG Fc binding protein		4321127	+	
1	Polymeric-Immunoglobulin receptor precursor	P01833		+	
1	Heat Shock 27kDa Protein	P04792		+	
1	Carbonic Anhydrase II	P00918		+	
1	Hypothetical protein PP1226		13775216	+	
1	ATP Synthase Alpha Chain, mitochondrial precursor	P25705		-	

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
1	3-hydroxyiso-buturyl-Coenzyme A hydrolase		13636047	-	
1	Aflatoxin B1 Aldehyde Reductase 2	O95154		-	
1	Acyl-CoA Dehydrogenase, medium chain specific	P11310		-	
1	Dihydrolipo-amide Dehydrogenase	P09622		-	
1	Fibrinogen Beta Chain Precursor	P02675		-	
1	Delta-1Pyrroline-5-Carboxylate Dehydrogenase Precursor	P30038		-	
1	Methylmalonate-Semialdehyde Dehydrogenase + Fibrinogen Beta Chain Precursor	Q02252 + P02675		-	two proteins present in the spot
1	Dead-Box protein 1	Q92499		-	
1	Peroxiderin 2	P02023		-	
1	Hydromethyl-glutaryl-CoA Synthase	P54868		-	
1	Alpha-Tryptase Precursor	P15157		-	
1	Alcohol Dehydrogenase	P14550		-	

Reproducibility	Name	Swissprot	NCBI	Expression	Comments
1	Acyl-CoA dehydrogenase 8		7656849	-	
1	Phosphoenol-pyruvate Carboxykinase, mitochondrial precursor	Q16822		-	
1	methylcrotonoyl-Coenzyme A carboxylase 1		13518228	-	
1	Triacylglycerol Lipase, Gastric precursor	P07098		-	
1	Barrier-to-autointegration factor	O75531		+	

	A	B	C	D	E	F	G	H	I
1	Accession #:	Name	Expression	Onco-publications	Gastric_cancer_publications	Public 2D db data	Patents_Depatis	Patents_esp@cenet	Onco-chip@NCBI
2	Q9Y4L1	150 kD Oxygen-regulated Protein Precursor	underexpressed	*Mol Urol*. 2001 5:79-80; *Cancer Res*. 2001 61:4206-13; *Lab Invest*. 1998 78:699-706; *Int J Radiat Oncol Biol Phys*. 1989 16:1301-5	no	Colon carcinoma cell line LIM1215	3 non-cancer	no	no
3	P11021	78 kDa Glucose regulated protein precursor	underexpressed/ overexpressed	*Electrophoresis* 1997 **18**:588; *Electrophoresis* 1997 **18**:605	no	Breast ductal carcinoma; Breast carcinoma cell line MDA-MB231; Colorectal carcinoma; Colon carcinoma cell line LIM1215	1 non-cancer	no	yes
4	OQ13966	Aconitate Hydratase, mitochondrial precursor	underexpressed	*J Biol Chem* 1994 269:29409-15	no		1 gene sequence		
5	P02570 P02571	Actin, Cytoplasmic 1 (beta-actin/gamma-actin)	overexpressed	no	no	Breast ductal carcinoma; Colorectal carcinoma; Colon carcinoma cell line LIM1215; Bladder Squamous Cell carcinoma; Transitional cell carcinoma		1 bladder-tumor	Only actin gamma 1
6	P11310	Acyl-CoA Dehydrogenase, medium chain specific	underexpressed	no	no	Bladder Squamous Cell carcinoma; Transitional cell carcinoma		no	no



	J	K	L	M	N
1	OMIM disease	Protein family	Function	Biological process	Splice_variants
2	no	HSP70	pivotal role in cytoprotective cellular mechanisms triggered by oxygen deprivation; may play a role as a molecular chaperone and participate in protein folding		
3	no	HSP70	probably monitors protein transport in ER	no data	
4					
5	no	actin family	cytoskeletal element	Cell structure	
6	dicarboxylic aciduria,non ketotic hypoglycemia,Reye-like syndrome,sudden infant death syndrome	acyl-coA dehydrogenase	oxidoreductase	Energy pathways/Fatty acid beta-oxidation	

	A	B	C	D	E	F	G	H	I
7	Accession #	Name	Expression	Onco-publications	Gastric_cancer_ publications	Public 2D db data	Patents_Depatis	Patents_esp@cenet	Onco-chip@NCBI
8	P16219	Acyl-CoA Dehydrogenase, Short Chain specific	underexpressed	no	no	no		no	no
9	P25705	ATP Synthase Alpha Chain, mitochondrial precursor	underexpressed	*Med Hypotheses* 2001 56:386-7; *Biochem Mol Biol Int* 1996 38:1013-22; *J Bioenerg Biomembr.* 1997 29:379-84 Review; *J Exp Med.* 1994 180:273-81	no	Colon carcinoma cell line LIM1215	no	no	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6
10	P06576	ATP Synthase Beta Chain, mitochondrial Precursor	underexpressed	*Med Hypotheses* 2001 56:386-7; *Biochem Mol Biol Int* 1996 38:1013-22; *J Bioenerg Biomembr.* 1997 29:379-84 Review; *J Exp Med.* 1994 180:273-81	no	Colon carcinoma cell line LIM1215	no	no	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F6
11	O75493	CA11(downregulated in gastric cancer)	underexpressed	no	Int J Oncolog 2001 19:701-707;Jpn J Cancer Res. 2000 91:459-63		no	no	
12	P27824	Calnexin Precursor	underexpressed	*Electrophoresis*. 1999 20:1065-74; *BBRC* 2001 289:725-32; *BBRC* 2001 286:1212-7; *Int J Oncol.* 2001 19:1211-20; *Bone Marrow Transplant.* 2000 May;25 Suppl 2:S88-95	no	Colon carcinoma cell line LIM1215; Bladder Squamous Cell carcinoma; Transitional cell carcinoma	not cancer-related	no	no
13	P04040	Catalase	underexpressed	*Cancer Res.* 2001 61:8578-85; *Histol Histopathol.* 1997 12:525-35. Review	no	Colon carcinoma cell line LIM1215; Transitional cell carcinoma		1 for tumor treatment	no

	J	K	L	M	N
7	OMIM disease	Protein family	Function	Biological process	Splice_variants
8	myopathy, lipid storage	acyl-coA dehydrogenase	oxidoreductase	Energy pathways/Fatty acid beta-oxidation	
9	no	alpha/beta ATPase family	F1 ATPase alpha subunit	Energy pathways/small molecule transport	
10	no	alpha/beta ATPase family	F1 ATPase beta subunit (ATP synthsis during oxidative phosphorylation	Energy pathways/small molecule transport	
11					
12	no	calreticulin family	involved in the secretion of proteins from the ER to the outer cellular membrane	Protein folding/protein translocation	
13	acatalasia	catalase family	oxidoreductase detoxifing hydrogen peroxides	Oxidative stress response	

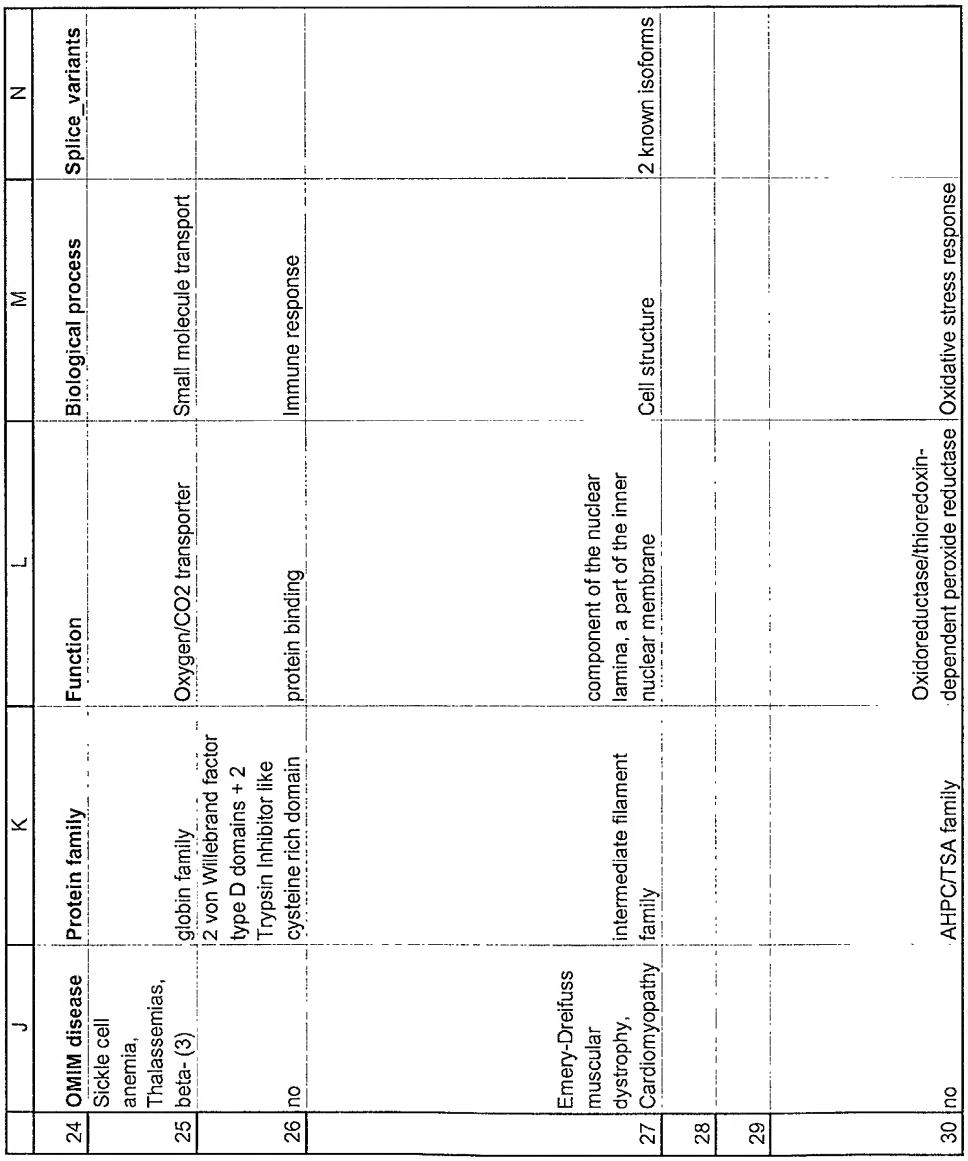
	A	B	C	D	E	F	G	H	I
14	Accession #	Name	Expression	Onco-publications	Gastric_cancer_ publications	Public 2D db data	Patents_Depatis	Patents_esp@cenet	Onco-chip@NCBI
15	P07339	Cathepsin D precursor	overexpressed	*J Gastroenterol*. 2001 36:809-15; *Rocz Akad Med Bialymst*. 200146:38-46; *Cancer*. 2001 92:2957-64; *Oncogene*. 2001 20:6920-9	*J Gastroenterol*. 2001 36:809-15	Breast ductal carcinoma; Bladder Squamous Cell carcinoma; Transitional cell carcinoma		2 for cancer prognosis	Only cathepsin B and L
16	P35900	Cytokeratin 20	underexpressed	*Ann Surg Oncol*. 2002 9:13-9; *Ann Surg*. 2002 235:226-31; *Med Oncol*. 2001 18:33-8; *Cancer*. 2001 92:2727-32	*Hum Pathol*. 2001 32:1087-93	Bladder Squamous Cell carcinoma; Transitional cell carcinoma	no_stomach_relat ed	3 for tumor detection	no
17	P08729	Cytokeratin 7	overexpressed	*Cancer*. 2001 92:2727-32; *Hum Pathol*. 2001 32:1087-93	*Hum Pathol*. 2001 32:1087-93	Transitional cell carcinoma	no_stomach_relat ed	no	no
18	P13716	Delta-Aminolevulinic Acid Dehydratase	underexpressed	*Blood*. 1985 65:939-44; *Comp Biochem Physiol B*. 1990 96:729-31; *Br J Cancer*. 1992 65:409-12	no	no	no	no	no

	J	K	L	M	N
14	OMIM disease	Protein family	Function	Biological process	Splice_variants
15	no	peptidase A1 family	acid protease	Protein degradation	
16	no	intermediate filament family	cytoskeletal element	Cell structure	
17	Cirrhosis	intermediate filament family	cytoskeletal element	Cell structure	
18	delta-aminolevulinate dehydratase deficiency, porphobilinogen synthase deficiency, acute hepatic porphyria	ALADH family	Lyase	Porphyrin and heme biosynthesis	

	A	B	C	D	E	F	G	H	I
19	Accession #	Name	Expression	Onco-publications	Gastric_cancer_ publications	Public 2D db data	Patents_Depatis	Patents_esp@cenet	Onco-chip@NCBI
20	P12830	Epithelial-Cadherin Precursor	underexpressed	*Cancer Treat Res*. 2002 107:305-29; *Gastroenterology*. 2002 122:331-339; *Cancer Lett*. 2002 176:123-8; *BMC Cancer*. 2001 1:16; *Br J Cancer*. 2001 85):1958-63	*BMC Cancer*. 2001 1:16	Bladder Squamous Cell carcinoma; Transitional cell carcinoma; Urina		1 for detecting predisposition to cancer	yes
21	P47756	F-Actin Capping Protein Beta subunit	overexpressed	*BBRC* 1986 134:1284-90	no	Bladder Squamous Cell carcinoma	1	no	no
22	P02675	Fibrinogen Beta Chain Precursor	underexpressed	*Dig Liver Dis*. 2001 33:546-50; *Thromb Res*. 2001 104:421-5	*Dig Liver Dis*. 2001 33:546-50	Breast ductal carcinoma	yes	no	no
23	P04792	Heat Shock 27 kDa Protein	overexpressed	*J Cancer Res Clin Oncol*. 2001 127:727-32; *Zhonghua Er Bi Yan Hou Ke Za Zhi*. 1998 33:232-4; *Int J Gynecol Cancer*. 1995 5:94-100; *Ann Oncol*. 2001 12:615-20; *J Endod*. 2001 27:89-92; *J Surg Res*. 2001 99:129-33; *Cancer Lett*. 2001 168:173-81; *Ann Surg Oncol*. 2001 8:215-21; *Mol Hum Reprod*. 1999 5:748-56	*Ann Surg Oncol*. 2001 8:215-21; *Zhonghua Er Bi Yan Hou Ke Za Zhi*. 1998 33:232-4	Transitional cell carcinoma	not cancer-related	2 for cancer detection	no

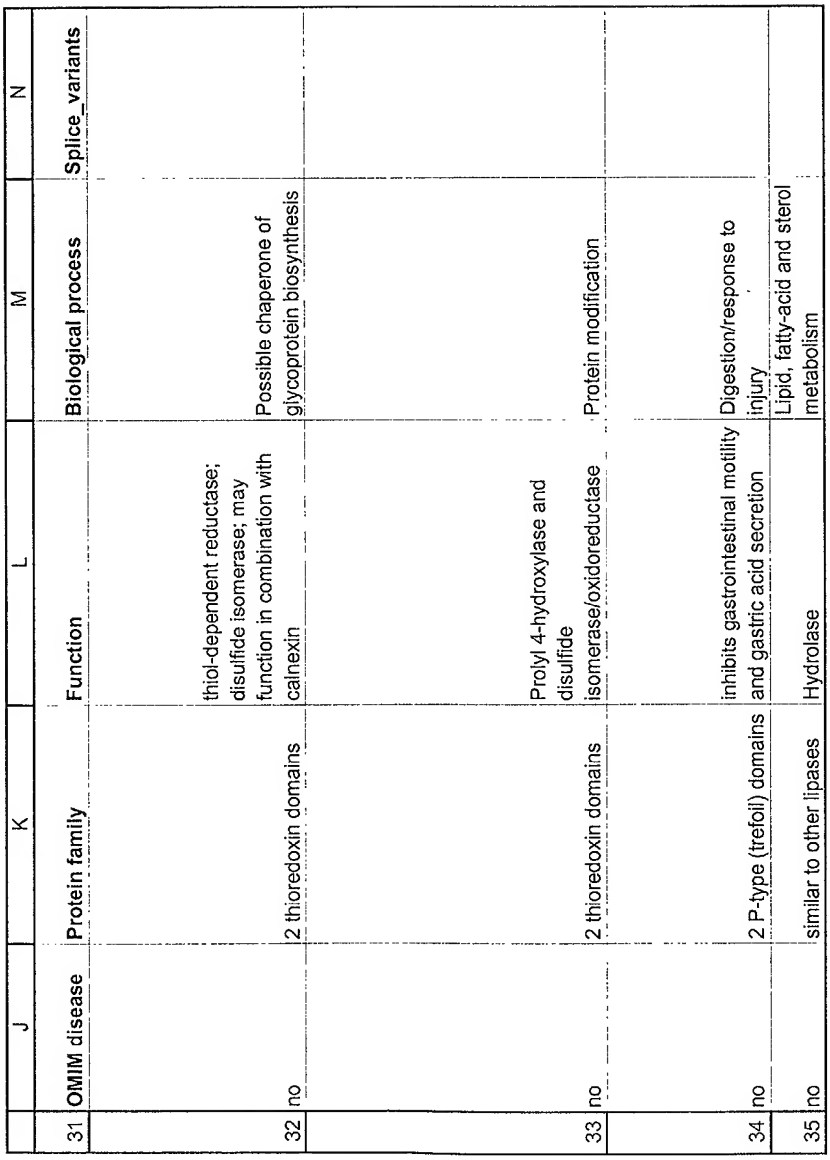
	J	K	L	M	N
19	**OMIM disease**	**Protein family**	**Function**	**Biological process**	**Splice_variants**
20	Endometrial carcinoma, Ovarian carcinoma, Breast cancer, Gastric cancer	5 cadherin domains	mediates cell-cell interactions in epithelial cells	Cell adhesion	
21	no	F-actin capping protein beta subunit family	actin-binding protein involved in actin assembly	Cell motility	3 known isoforms
22	Dysfibrinogenemia beta type, Afibrinogenemia, congenital	1 fibrinogen domain	cofactor in platelet aggregation	blood coagulation; blood pressure regulation; positive control of cell proliferation; response to wounding	
23	no	HSP20	associates with alpha- and beta-tubulin and microtubules	stress resistance and actin organization	

	A	B	C	D	E	F	G	H	I
24	Accession #	Name	Expression	Onco-publications	Gastric_cancer_ publications	Public 2D db data	Patents_Depatis	Patents_esp@cenet	Onco-chip@NCBI
25	P02023	Hemoglobin Beta Chain	underexpressed	no	no	Breast ductal carcinoma; Colorectal carcinoma		no	Only Hemoglobin alpha 1
26	AC006950	IgG Fc binding protein 99% identical to D84239	underexpressed/ overexpressed	*J Biol Chem* 1997 272:15232-41	no	no		no	Only Fc fragment of IgE and IgG
27	P02545	Lamin A/C	underexpressed/ overexpressed	*Int J Oncol.* 2001 19:1325-32; *J Biol Chem.* 2002 277:804-15; *Br J Cancer.* 2001 84:512-9; *Histol Histopathol.* 2001 16:1-14; *J Cell Biochem.* 1999;Suppl 32-33:183-91. Review; *Exp Neurol.* 1999 157:241-50; *Biochem Pharmacol.* 1999 57:143-54	*Gut.* 1999 45:723-9	no	no	no	no
28	Q01828	Nucleobindin 1 precursor	underexpressed	*Pathol Int.* 1998 48:22-8	no	no	no		
29	Q9N2D4	Pepsin A precursor	underexpressed		*Neoplasma* 1992 39:107-14;				
30	P32119	Peroxiredoxin 2	underexpressed	*Anticancer Res.* 2001 21:2085-90; *Anticancer Res.* 2001 21:1129-33; *Clin Cancer Res.* 2000 6:4915-20; *Electrophoresis* 1997 18:605-613	no	no	no	no	no



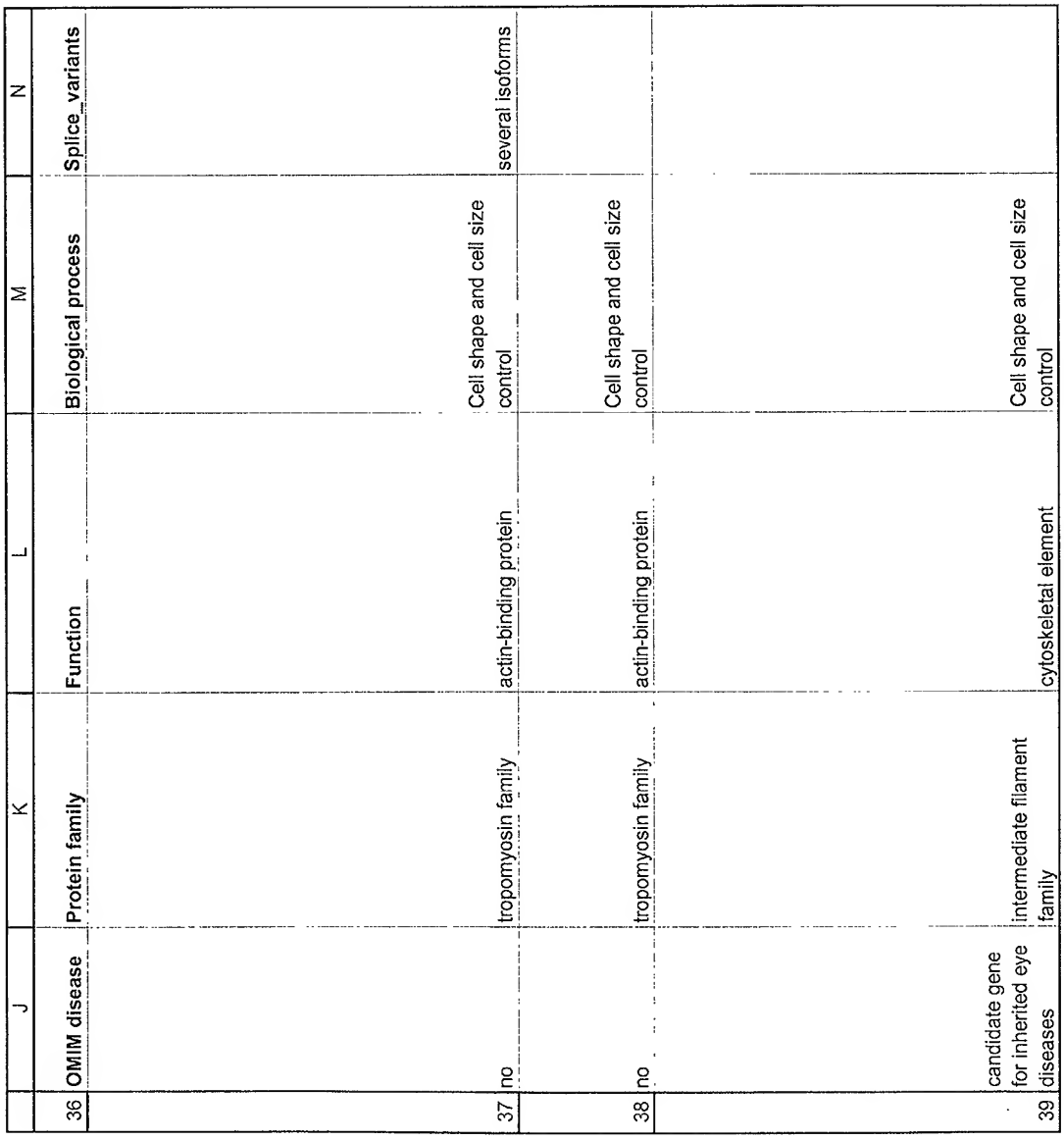
	J	K	L	M	N
24	**OMIM disease**	**Protein family**	**Function**	**Biological process**	**Splice_variants**
25	Sickle cell anemia, Thalassemias, beta- (3)	globin family	Oxygen/CO2 transporter	Small molecule transport	
26	no	2 von Willebrand factor type D domains + 2 Trypsin Inhibitor like cysteine rich domain	protein binding	Immune response	
27	Emery-Dreifuss muscular dystrophy, Cardiomyopathy	intermediate filament family	component of the nuclear lamina, a part of the inner nuclear membrane	Cell structure	2 known isoforms
28					
29					
30	no	AHPC/TSA family	Oxidoreductase/thioredoxin-dependent peroxide reductase	Oxidative stress response	

	A	B	C	D	E	F	G	H	I
31	Accession #	Name	Expression	Onco-publications	Gastric_cancer_publications	Public 2D db data	Patents_Depatis	Patents_esp@cenet	Onco-chip@NCBI
32	P30101	Protein Disfide Isomerase A3 Precursor=GRP58 (58 kDa glucose-regulated protein	underexpressed	*Electrophoresis* 1997 18:605; *Electrophoresis* 1997 18:588; Europ. J. Biochem. 1995 234:336-342; J Biol Chem. 1999 274:25499-509	no	Breast ductal carcinoma; Breast carcinoma cell line MDA-MB231; Colon carcinoma cell line LIM1215; Bladder Squamous Cell carcinoma; Transitional cell carcinoma	no	no	no
33	P07237	Protein Disulfide Isomerase Precursor	underexpressed/ overexpressed	*Electrophoresis* 1997 18:605; J Histochem Cytochem. 2001 49:1143-53; Anticancer Res. 2000 20:4733-8; J Surg Oncol. 1999 70:239-46; J Pathol. 2000 191:376-86; J Cell Biochem. 1997 65:542-9; Am J Surg Pathol. 1998 22:1393-403	*Anticancer Res.* 2000 20:4733-8; *J Surg Oncol.* 1999 70:239-46	Breast ductal carcinoma; Bladder Squamous Cell carcinoma; Transitional cell carcinoma	no	no	no
34	Q03403	Spasmolytic Polypeptide Precursor	underexpressed	*FASEB J.* 2001 15:351-61; *Dig Dis Sci.* 2000 45:659-64; *PNAS* 1999 96:2316-21; *J Pathol.* 1997 183:4-7. Review; *EMBO J.* 1990 9:407	*J Pathol.* 1997 183:4-7 ?	no	1 non+ 1 carcinoma screen	2 for screen for risk for gastric adenocarcinoma	no
35	P07098	Triacylglycerol Lipase, gastric precursor	underexpressed	no	*J Cell Biochem.* 2001 81:241-51	no		no	no



	J	K	L	M	N
31	OMIM disease	Protein family	Function	Biological process	Splice_variants
32	no	2 thioredoxin domains	thiol-dependent reductase; disulfide isomerase; may function in combination with calnexin	Possible chaperone of glycoprotein biosynthesis	
33	no	2 thioredoxin domains	Prolyl 4-hydroxylase and disulfide isomerase/oxidoreductase	Protein modification	
34	no	2 P-type (trefoil) domains	inhibits gastrointestinal motility and gastric acid secretion	Digestion/response to injury	
35	no	similar to other lipases	Hydrolase	Lipid, fatty-acid and sterol metabolism	

	A	B	C	D	E	F	G	H	I
36	Accession #	Name	Expression	Onco-publications	Gastric_cancer_ publications	Public 2D db data	Patents_Depatis	Patents_esp@cenet	Onco-chip@NCBI
37	P06468	Tropomyosin Beta Chain, Fibroblast and Epithelial	overexpressed	*Mol Carcinog*. 2002 33:9 15; *Cell Growth Differ*. 2001 12:543-50; *Oncogene*. 2001 20:2112-21; *J Biol Chem*. 2001 276:25190-6; *Anal Cell Pathol*. 2000 21:1-9; *Electrophoresis*. 2000 21:1210-7. Review; *PNAS* 2000 97:3260-5; *Hybridoma*. 1999 18:543-6; *Somat Cell Mol Genet*. 1998 24:273-80; *Cell Growth Differ*. 1998 9:565-73	*Hybridoma*. 1998 17:431-5	Transitional cell carcinoma	no	no	no
38	P07226	Tropomyosin, Fibroblast non-muscle type (tropomyosin 4)	overexpressed	*J Biol Chem*. 2001 276:47421-33; *Blood*. 2001 98:1209-16; *Electrophoresis*. 1999 20:241-8	*Genomics*. 2000 69:281-6; *Hybridoma*. 1998 17:431-5	Bladder Squamous Cell carcinoma; Transitional cell carcinoma		no	no
39	P08670	Vimentin	overexpressed	*Mol Hum Reprod*. 1999 5:748-56; *Endocr Relat Cancer*. 2000 7:199-226. Review; *Virchows Arch*. 2001 439:768-75. Review; *Electrophoresis* 1997 18:588	*Surg Today*. 2001 31:346-9; *J Clin Gastroenterol*. 1999 29:82-5; *Pathol Res Pract*. 1999 195:841-6; *Am J Surg Pathol*. 1999 23:431-6; *Hum Pathol*. 1997 28:1355-60; *Am J Clin Pathol*. 1997 108:641-51	Breast carcinoma cell line MDA-MB231; Transitional cell carcinoma	no stomach-related	no	yes



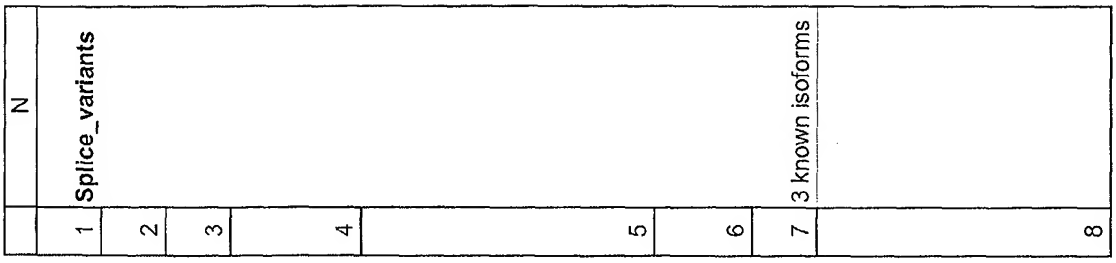
	J	K	L	M	N
36	OMIM disease	Protein family	Function	Biological process	Splice_variants
37	no	tropomyosin family	actin-binding protein	Cell shape and cell size control	several isoforms
38	no	tropomyosin family	actin-binding protein	Cell shape and cell size control	
39	candidate gene for inherited eye diseases	intermediate filament family	cytoskeletal element	Cell shape and cell size control	

	A	B	C	D	E	F	G	H	I
40	Accession #	Name	Expression	Onco-publications	Gastric_cancer_ publications	Public 2D db data	Patents_Depatis	Patents_esp@cenet	Onco-chip@NCBI
	P45880								
41		Voltage-Dependent Anion-Selective Channel protein 2	underexpressed	*Biol Chem Hoppe Seyler*. 1992 373:891-6; *J Bioenerg Biomembr*. 1994 26:317-25 Review; *Eur J Biochem*. 2000 267:6067-73; *J Cell Biol*. 2001 155:1003-15	no	Colon carcinoma cell line LIM1215	no	1 for cancer treatment	no

	J	K	L	M	N
40	OMIM disease	Protein family	Function	Biological process	Splice_variants
41	no	eukaryotic mitochondrial porin family	mitochondrial membrane channel protein and binding site of the metabolic enzymes hexokinase and glycerol kinase to the mitochondrion	small hydrophilic molecules transport	4 known isoforms

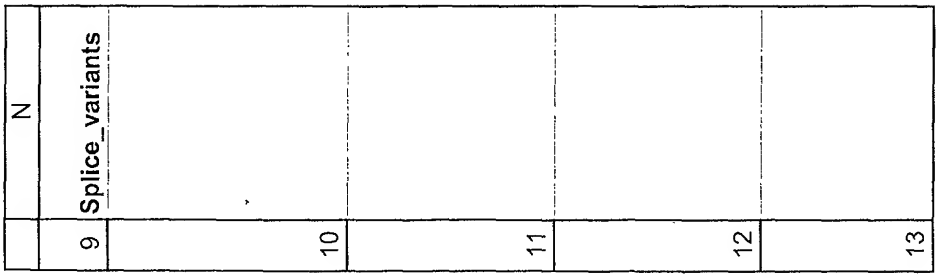
	A	B	C	D	E	F	G
1	**Accession #**	**Name**	**Expression**	**Onco-publications**	**Gastric_cancer_ publications**	**Public 2D db data**	**Patents_Depatis**
2	Q14259	Enhancer of rudimentary homolog	overexpressed	no	no	no	no
3	O00151	PDZ and LIM Domain Protein 1	overexpressed	no	no	no	no
4	P55072	Transitional Endolasmatic Reticulum ATPase	overexpressed	*DNA Cell Biol*. 2000 19:253-63	no	no	no
5	P20142	Gastricsin (pepsinogen C or II)	underexpressed	*Int J Biol Markers*. 2001 16:240-4; *Gut*. 2001 49:335-40; *Br J Cancer*. 2001 85:228-34; *Int J Biol Markers*. 2001 16:31 *Nippon Rinsho*. 6; *J Cell Biochem*. 2001 81:241-51 *Cancer Detect Prev*. 2000 24:564-71	2001 59 Suppl 4:204-7; *Nippon Rinsho*. 2001 59 Suppl 4:538-42	Urina	2 non-cancer
6	P02749	Beta-2-Glycoprotein I Precursor (apolipoprotein H)	underexpressed	*J Biochem* (Tokyo). 2000 128:1017-24	no	no	no
7	P47756	F-Actin Capping Protein Beta subunit	overexpressed	*BBRC* 1986 134:1284-90	no	Bladder Squamous Cell carcinoma	1
8	P27824	Calnexin Precursor	underexpressed	*Electrophoresis*. 1999 20:1065-74; *BBRC* 2001 289:725-32; *BBRC* 2001 286:1212-7; *Int J Oncol*. 2001 19:1211-20; *Bone Marrow Transplant*. 2000 May;25 Suppl 2:S88-95	no	Colon carcinoma cell line LIM1215; Bladder Squamous Cell carcinoma; Transitional cell carcinoma	not cancer-related

	H	I	J	K	L	M
1	**Patents_esp@cenet**	**Onco-chip@NCBI**	**OMIM disease**	**Protein family**	**Function**	**Biological process**
2	no	no	no	E(R) family	potential cell cycle regulator	no data
3	no	no	no	1 PDZ and 1 LIM domains	cytoskeletal protein	Oxidative stress response
4	no	no	no	AAA family of ATPases	involved in membrane trafficing from ER to Golgi and ubiquitin-proteasome degradation protease	Vesicle transport/Protein degradation
5	no	no	no	peptidase A1 family		Protein degradation during digestion
6	no	no	no	4 SUCHI domains	Apolipoprotein H	Immune response
7	no	no	no	F-actin capping protein beta subunit family	actin-binding protein involved in actin assembly	Cell motility
8	no	no	no	calreticulin family	involved in the secretion of proteins from the ER to the outer cellular membrane	Protein folding/protein translocation


N
Splice_variants
3 known isoforms
1
2
3
4
5
6
7
8

	A	B	C	D	E	F	G
9	**Accession #**	**Name**	**Expression**	**Onco-publications**	**Gastric_cancer_ publications**	**Public 2D db data**	**Patents_Depatis**
10	P32119	Peroxiredoxin 2	underexpressed	*Anticancer Res.* 2001 21:2085-90; *Anticancer Res.* 2001 21:1129-33; *Clin Cancer Res.* 2000 6:4915-20; *Electrophoresis* 1997 18:605-613	no	no	no
11	P11021	78 kDa Glucose regulated protein precursor	underexpressed/ overexpressed	*Electrophoresis* 1997 **18**:588; *Electrophoresis* 1997 **18**:605	no	Breast ductal carcinoma; Breast carcinoma cell line MDA-MB231; Colorectal carcinoma; Colon carcinoma cell line LIM1215	1 non-cancer
12	Q9Y4L1	150 kD Oxygen-regulated Protein Precursor	underexpressed	*Mol Urol.* 2001 5:79-80; *Cancer Res.* 2001 61:4206-13; *Lab Invest.* 1998 78:699-706; *Int J Radiat Oncol Biol Phys.* 1989 16:1301-5	no	Colon carcinoma cell line LIM1215	3 non-cancer
13	O75493	CA11(downregulated in gastric cancer)	underexpressed	no	Int J Oncolog 2001 19:701-707;Jpn J Cancer Res. 2000 91:459 63		no

	H	I	J	K	L	M
9	Patents_esp@cenet	Onco-chip@NCBI	OMIM disease	Protein family	Function	Biological process
10	no	no	no	AHPC/TSA family	Oxidoreductase/thioredoxin-dependent peroxide reductase	Oxidative stress response
11	no	yes	no	HSP70	probably monitors protein transport in ER	no data
12	no	no	no	HSP70	pivotal role in cytoprotective cellular mechanisms triggered by oxygen deprivation; may play a role as a molecular chaperone and participate in protein folding	
13	no					



Claims

1. Use of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P20142 Gastricsin, P07492 Beta-2-Glycoprotein I Precursor, P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor, 075493 CA11, nucleic acid sequences encoding said proteins and/or recognition agents for said proteins or said nucleic acid sequences for the manufacture of an agent for diagnosis, prophylactic or therapeutic treatment of non-steroid dependent cancer.

2. A Method for detecting non-steroid dependent cancer cells in a sample from a patient, wherein the method comprising providing the sample and detecting the level of one or more proteins selected from the group consisting of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P20142 Gastricsin, P07492 Beta-2-Glycoprotein I Precursor, P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11 in the sample, and comparing the level of the one or more proteins with a control level that is representative of a level in a normal, cancer-free patient, wherein an modified level of the proteins in the sample compared to the level of the proteins in the control sample indicates the presence of non-steroid dependent cancer in the patient.

3. The method of claim 2 wherein the proteins are detected by determining the copy number of nucleic acids encoding for the proteins, the expression level of a gene or genes encoding the protein and/or by measuring the level of the proteins or protein activity in the sample.

4. The method according to claim 2 or 3, wherein the method comprising contacting the sample with a recognition agent for one or more of the proteins or nucleic acid sequences encoding said proteins and detecting binding of the recognition agent to at least one of the proteins or the nucleic acid sequences in the prepared sample as an indication of the presence of cancer cells in the sample.

5. The method of claim 2 to 4, wherein binding of the recognition agent to one or more of the proteins is detected by immunoblotting or immunohistochemical analysis, radioimmunoassay, Western blot analysis or enzyme labelling technique.

6. Method of treatment of non-steroid dependent cancer, **characterised by** a modification of the level of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P20142 Gastricsin, P07492 Beta-2-Glycoprotein I Precursor, P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor, 075493 CA11, nucleic acid sequences encoding said proteins and/or recognition agents for said proteins or said nucleic acid sequences.

7. Method according to claim 6, **characterised by** a reduction of levels of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P47756 F-Actin Capping Protein Beta subunit and/or P11021 78 kDa Glucose regulated protein.

8. Method according to claim 7, **characterised by** down-regulation of promoters of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P47756 F-Actin Capping Protein Beta subunit and/or P11021 78 kDa Glucose regulated protein.

9. Method according to claim 6, **characterised by** a raising of levels of P20142 Gastricsin, P07492 Beta-2-Glycoprotein I Precursor, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11.

10. Method according to claim 9, **characterised by** up-regulation of promoters of P20142 Gastricsin, P07492 Beta-2-Glycoprotein I Precursor, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor and/or 075493 CA11.

11. A kit for detection of cancer cells in a sample comprising at least one protein selected from the group consisting of Q14259 Enhancer of rudimentary homolog, 000151 PDZ and LIM Domain Protein 1, P55072 Transitional Endolasmatic Reticulum ATPase, P20142 Gastricsin, P07492 Beta-2-Glycoprotein I Precursor, P47756 F-Actin Capping Protein Beta subunit, P27824 Calnexin Precursor, P32119 Peroxiredoxin 2, P11021 78 kDa Glucose regulated protein precursor, Q9Y4L1 150 kD Oxygen-regulated Protein Precursor, 075493 CA11, nucleic acid sequences encoding said proteins and/or recognition agents for said proteins or said nucleic acid sequences.

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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 02 09 0236

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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TECHNICAL FIELDS SEARCHED (Int.Cl.7)

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C12Q
A61K
G01N
C12N

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	21 January 2003	GONCALVES M L F C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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INCOMPLETE SEARCH SHEET C

Application Number
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Claims 6-10 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), thus no search was carried out for the subject-matter of these claims.

Claim(s) searched completely:
1-5,11

Claim(s) not searched:
6-10

Reason for the limitation of the search (non-patentable invention(s)):

Article 52 (4) EPC - Method for treatment of the human or animal body by therapy

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 02 09 0236

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The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82